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Mesenchymal Stem Cell Based Intradiscal Gene Therapy: Therapeutic Implication in Degenerative Disc Disease

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– Abstract –

Study Design: In-vitro experiments using human mesenchymal stem cells (MSCs), intervertebral disc (IVD) cells and type 5 adenovirus/transforming growth factor- β 1 construct (Ad/TGF- β 1).

Objectives: To determine the effect of MSC-based gene therapy for matrix regeneration of IVD cells.

Summary of Literature Review: MSCs are known to be multipotent in tissue regeneration. In degeneration of IVD, cellular replacement with genetic modification other than that of IVD cells may prove an enhanced mechanism for the regeneration of IVD cells.

Materials and Methods: MSCs and IVD cells were cultured and an adenovirus construct containing TGF- β 1 cDNA (Ad/TGF- β 1) was also produced. In the first step, the MSCs were transduced with Ad/TGF- β 1, then mixed with IVD cells in various proportions and three dimensionally cultured. [methyl- 3 H]Thymidine and [35 S]Sulfur incorporation for DNA and proteoglycan synthesis, respectively, were measured. RT-PCR was performed to assess the aggrecan and collagen types I and II mRNA expressions.

Results: Mixed cultures of MSC and IVD cells showed relatively similar amounts of newly synthesized proteoglycan compared with cultures of IVD cells only. In mixed cultures transduced with Ad/TGF- β 1, there were significant decreases in newly synthesized proteoglycan with increasing the proportions of MSCs, which was also found with the aggrecan and collagen type II mRNA expressions. However, the collagen type I mRNA expression increased with increased proportions of MSCs transduced with Ad/TGF- β 1.

Conclusion: Cell therapy with MSCs and IVD cells provided a mechanism for cellular augmentation. However, MSC-based gene therapy coupled with IVD cells did not maintain a chondrogenic phenotype.

Key words: Mesenchymal stem cell, disc cell, TGF- β 1, adenovirus, gene therapy

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(, , , ,)

가 ¹⁾ .

(proteoglycan) .

2 (type II collagen)

(nucleus pulposus) ²⁻⁸⁾ , .

⁹⁾ .

, , , 가 ,

, ^{8,10-11)} , ,

가 ²⁵⁻³⁰⁾ .

가

(trans-
forming growth factor- β 1, osteogenic protein-1, insulin
like growth factor-1) ,

¹²⁻¹⁶⁾ , ,

(matrix) .

가

1998 Nishimura¹⁶⁾ 가

, Okuma¹⁷⁾ ,

,

가 .

1.

, , 5

¹⁸⁻²⁰⁾ ,

(vector) ,

²¹⁻²⁴⁾ .

grade III, IV

20

Dulbecco's Phosphate-Buffered saline (D-PBS, Invitrogen, Grand Island, NY)

5% (FBS, JRH BIOSCIENCES, Lenexa, KS), 0.2% pronase (Sigma, St. Louis, MO), 0.004% deoxyribonuclease II type IV (DNase, Sigma, St. Louis, MO) Ham's F-12 medium (Invitrogen, Grand Island, NY)

37 60 D-PBS pronase 0.02% collagenase type II (Worthington Biochemical Corp., Lakewood, NJ) 2 37 °C 4

Ham's F-12 medium and Dulbecco's Modified Eagle Medium (DMEM/F12, Invitrogen, Grand Island, NY) nylon (pore size 75 µm)

5 × 10⁵ cells/ml 25 cm²-EasYFlask™ (Nunc, Rockville, Denmark)

10% FBS, 25 µg/ml ascorbic acid, 1% v/v penicillin, streptomycin, nystatin (all antibiotics from Invitrogen, Grand Island, NY) DMEM/F12

3 5% CO₂ 37 °C 3

2.

10ml 10% FBS Low glucose-Dulbecco's Minimal Essential Medium (DMEM-LG, Invitrogen, Grand Island, NY) 20 ml

10 ml Ficoll-Paque™ PLUS (Amersham pharmacia, Uppsala, Sweden)

25 cm²-EasYFlask™ (Nunc, Rockville, Denmark) 5% CO₂

37 °C 5 가

3 가

3.

E1 E3 5

Luciferase (Ad/luc), TGF-1 (Ad/TGF-1) cytomegalovirus promotor 293 , CsCl , 1 × 10¹² particles/ml 1.5 ml tube -70 °C particle

가 particle 1:100

4.

3 3 , 0.15M NaCl 2.4% low viscosity alginic acid (Sigma, St. Louis, MO) 1/2 alginic acid

가 1.2%가 2 × 10⁶ cells/ml 26 G 1cc , 24well plate 102 mM CaCl₂ alginic acid

well 10 가 polymerization 0.15 M NaCl

10 10% FBS, 25 µg/ml ascorbic acid, 1% v/v penicillin, streptomycin (all antibiotics from Invitrogen, Grand Island, NY) DMEM/F12

well 1 ml 가 5% CO₂ 37 °C 가 7

5.

PBS , 5% FBS가 DMEM-LG , 가

Ad/luc 가 Ad/TGF-1 5% FBS가 DMEM-LG 가

100% 150MOI가 가

가 5% CO₂ 37 °C 24 가 24

Table 1. Primer sequence and reaction temperature

	primer sequence (5'→3')	annealing temperature (°C)
-actin	GGC GGA CTA TGA CTT AGT TG AAA CAA CAA TGT GCA ATC AA	53
aggrecan	GGA TCT AGC AGT GAG ACG TC CTG CAG CAG TTG ATT CTG AT	47
collagen type I	CCT GTC TGC TTC CTG TTA AC AGA GAT GAA TGC AAA GGA AA	48
collagen type II	CAG GAC CAA AGG GAC AGA AA TTG GTC CTT GCA TTA CTC CC	54

- cells/ml, 0.25% Trypsin, 1 mM EDTA, 4 × 10⁶, 1:0, 0:1, 1:1, 1:4 가, alginic acid 3, PD-10 column (Amersham pharmacia, Uppsala, Sweden), Liquid scintillation cocktail (Beckman, Fullerton, CA) 6 ml 가, 16, 2, 3, 4, -scintillation counter (Packard, Downers Grove, IL)
6. 3, [methyl-³H]Thymidine (Amersham pharmacia, Uppsala, Sweden) 5 µCi/ml 가, 24, 24, cell harvester, Glass microfibre filter (Whatman, Maidstone, England), D-PBS, unbounded [methyl-³H]Thymidine, 16, membrane, membrane, scintillation vials, Liquid scintillation cocktail (Beckman, Fullerton, CA) 3 ml 가, 16, DNA가, -scintillation counter (Packard, Downers Grove, IL), DNA
7. 3, [³⁵S]-Sulfur (Amersham pharmacia, Uppsala, Sweden) 20 µCi/ml 가, 4 가, 8M guanidine hydrochloride, 20 mM EDTA, proteinase inhibitors 가, 4 °C, 48, Sephadex G-25M
8. (Aggrecan, collagen type I, type II mRNA) 3, bead, 28 mM EDTA (pH8.0), 0.15M NaCl 가, depolymerization, RNeasy mini kit (QIAGEN, Maryland, USA), RNA, RNA 1 µg, Oligo d(T)16 primer 2.5 µM (Invitrogen, Grand Island, NY) 가, 70 °C, 5, annealing, RT premix (Bioneer, ,) 42 °C, 1, 95 °C, 5, 4 °C, 5, cDNA, cDNA 1 ul, primer 10 pmol/ul 가, 가 20 ul가, PCR premix (Bioneer, ,), PCR (Table 1). RT-PCR, internal control, -actin, TINA program
9. SPSS (SPSS Inc. Chicago IL), One-way ANOVA, Fisher's protected LSD post-hoc test, p<0.05

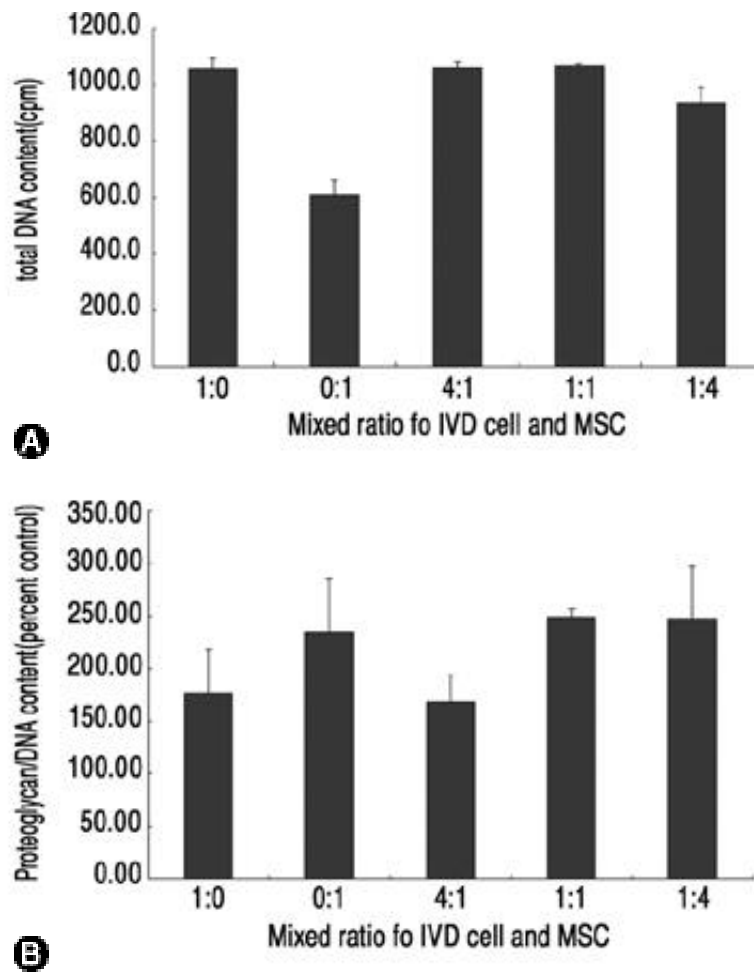


Fig. 1. (A) total DNA content in three dimensional culture of IVD cells and MSCs, (B) Newly synthesized proteoglycan in three dimensional culture of IVD cells and MSCs presented as percent control. Mixed cultures with IVD cells and MSCs equalled or quite decreased in newly synthesized proteoglycan comparing only IVD cell cultures (p 0.05).

가 가 ,

1. (Fig. 1B).

Ad/TGF- 1 가

, 7 3

(Fig. 1A), Ad/TGF- 1 30 50% , Ad/TGF- 1가 60 70%

가 (Fig. 2B). DNA

(p<0.05, Fig. 2A).

2. 3

3 ,

aggrecan, collagen

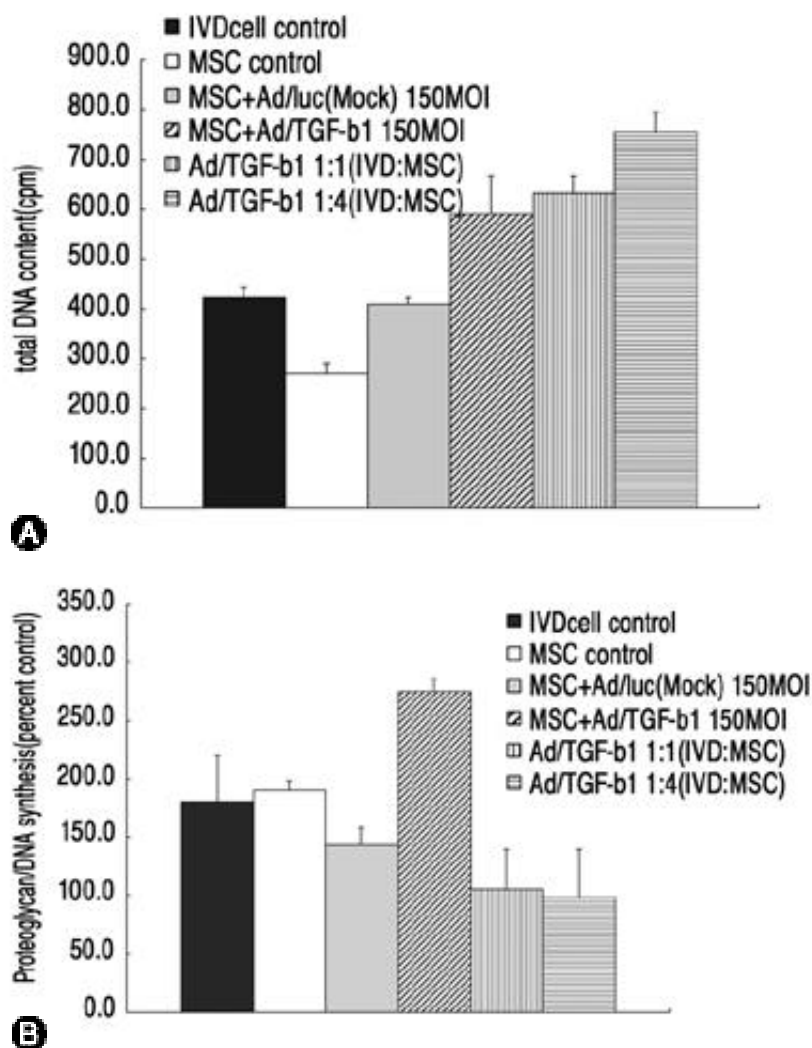


Fig. 2. (A) total DNA content in three dimensional culture of IVD cells and Ad/TGF- 1-transduced MSCs, (B) Newly synthesized proteoglycan in three dimensional culture of IVD cells and Ad/TGF- 1-transduced MSCs presented as percent control (p 0.05).

type I, collagen type II mRNA
, 50 70%

, 가 , 2
mRNA (collagen type II)
(Fig. 3). 1 (collagen type I)

Ad/TGF- 1

, collagen type I mRNA

가 가 (aggrecan, collagen type II) 가
(Fig. 4).

1991 Thompson ¹²⁾

TGF- 1

가

, Gruber ¹⁴⁾

TGF- 1

3

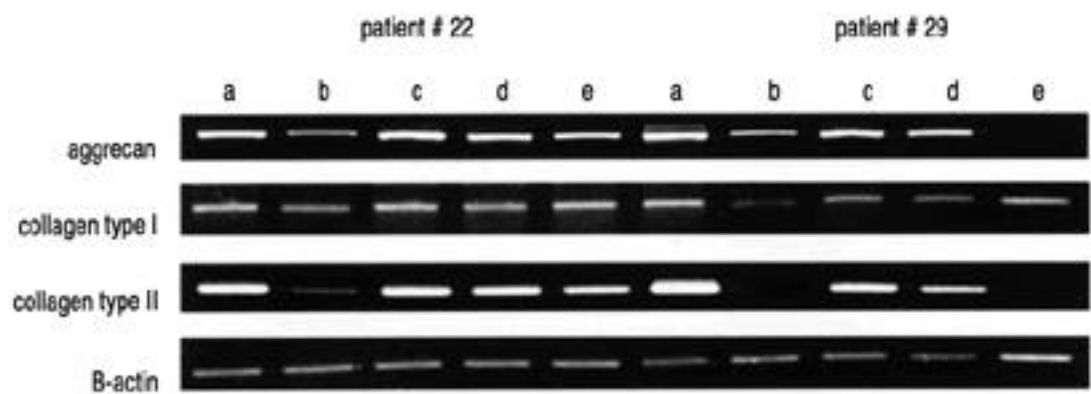


Fig. 3. RT-PCR for aggrecan, collagen type I, and collagen type II in three dimensional culture of IVD cells and MSCs. -actin was used for normalization. Mixed ratio of IVD cells and MSCs are a) 1:0, b) 0:1, c)4:1, d) 1:1, e) 1:4.

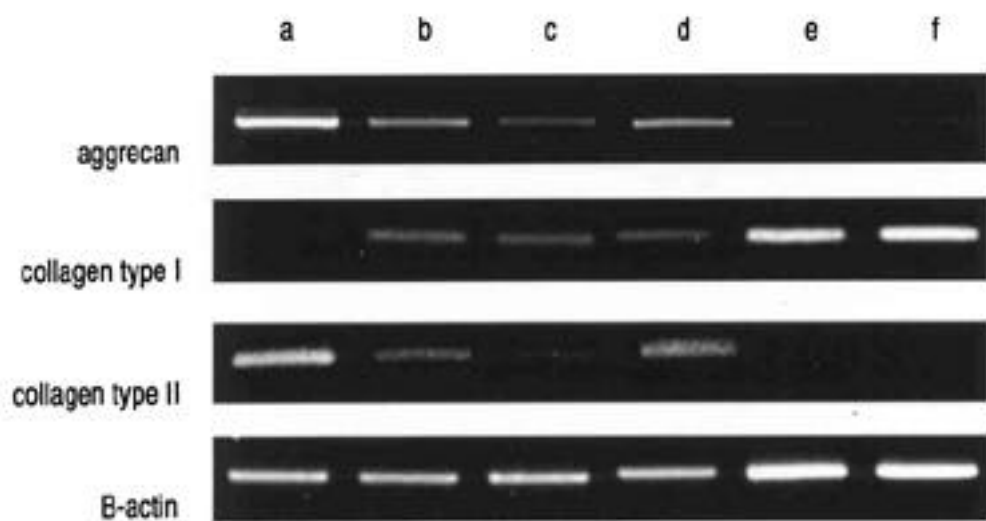


Fig. 4. RT-PCR for aggrecan, collagen type I, and collagen type II in three dimensional culture of IVD cells and Ad-transduced MSCs. -actin was used for normalization. a) only IVD cells, b) only MSC, c) only Ad/luc-transduced MSC [Mock], d) only Ad/TGF- 1-transduced MSC [Ad/TGF- 1-MSC], e) [IVD cells : Ad/TGF- 1-MSC] 1:1, f) [IVD cells : Ad/TGF- 1-MSC] 1:4.

가

IGF-1, BMP-2

가

35)

가

31-34)

가

가

가

3

가

TGF- 1

가

TGF- 1

RT-PCR

가 aggrecan collagen

collagen type I

mRNA 가 TGF- 1

가

가

가

가

2001 Hanada ³⁶⁾

3 TGF- 1

collagen type II mRNA

BMP-2

3

TGF- 1 BMP-2

BMP-2

TGF- 1 cDNA

TGF- 1 cDNA

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 : TGF- 1-
 :
 TGF- 1 TGF- 1 가 5 (Ad/TGF- 1)
 TGF- 1 가 alginate 3 . [methyl-³H] Thymi-
 dine , [³⁵S]Sulfur
 RT-PCR densitometry .
 : ,
 . Ad/TGF- 1 ,
 type II 가 Aggrecan collagen
 collagen type I 가
 가 .
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 ,
 : , TGF- 1, ,

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